Hybrid proteins for the screening of transcription regulators of reductive dehalogenase gene clusters

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In the past decade, research on the transcription regulation of reductive dehalogenase (*rdh*) gene clusters (for a recent review, see [1]) revealed a new set of CRP/FNR-type transcription regulators, called RdhK [2,3]. Several members of this family have been characterized in *Desulfitobacterium hafniense*. CprK1 (also known as RdhK6 [3]) is responsible for the regulation of the chlorophenol *rdh* operon [4-5]. The binding of the CprK1 N-terminal domain to 3-chloro-4-hydroxyphenylacetate (effector) promotes C-terminal specific interaction with a DNA target (dehalobox) in the operon promoter which leads to transcription. The presence of 25 copies of *rdhK* genes in *Dehalobacter restrictus* suggests an important regulation network of organohalide respiration. Moreover, the proximity of *rdhK genes in rdh* gene clusters [6] offers an indirect way to identify the substrate of yet uncharacterized RdhA enzymes.

Overall this study aims to identify effectors and dehaloboxes recognized by *D. restrictus* RdhK regulators. Electrophoretic mobility shift assay (EMSA) has been used to characterize CprK1 and other RdhK [5] and still represents a key methodology to detect tri-partite interactions. However, the main challenge resides in the large number of combinations of effectors and dehaloboxes to test for each new RdhK protein. To circumvent this difficulty, the strategy developed here is to use hybrid proteins made by the N-domain from a characterized RdhK, such as CprK1, fused to the C-domain from one of a yet uncharacterised RdhK from *D. restrictus*, or vice-versa. This strategy will be applied to screen either for the dehalobox or for the effector of the uncharacterised regulatory protein, respectively.

So far, as proof of concept, a protein hybrid, RdhK-N61C, was created by fusing *D. hafniense* RdhK6 and RdhK1 domains. Preliminary data show that this RdhK hybrid keeps specific binding activities for the effectors and dehaloboxes of their corresponding domains. Initial investigation with *D. restrictus* RdhK domains will also be discussed.

References:

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